Porin new light onto chromatin and nuclear organization

Minireview

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Abstract

A recent report identifies sites in the human genome that can associate with nucleoporin 93, a subunit of the nuclear pore complex. These associations are modulated by levels of global histone acetylation and highlight the dynamic nature of chromatin organization in the nucleus.

Nuclear pore complexes (NPCs) in the nuclear envelope mediate the selective exchange of macromolecules into and out of the nucleus by facilitated diffusion. The NPC is composed of multiple copies of roughly 30 distinct nucleoporins [1-3] and its structure is conserved throughout eukaryotes. Although numerous studies have examined how nucleoporins function in nucleo-cytoplasmic transport, relatively little attention has been paid to any role they might have in influencing genome organization in mammalian cells.

The nuclear periphery has customarily been associated with inactive chromatin structure and with the repression of gene expression [4-7]. Evidence for a more complex relationship between gene activity and the nuclear periphery, linking the upregulation of gene expression with the association of chromatin with the NPC, came initially from studies in budding yeast (*Saccharomyces cerevisiae*) and *Drosophila* [8,9]. Now, a recent report in *Genes & Development* from Pamela Silver and her colleagues (Brown *et al.* [10]) shows, by chromatin immunoprecipitation (ChIP), that physical associations between the nucleoporin NUP93 and specific regions of human chromosomes can be captured, and moreover that the inhibition of histone deacetylases (HDACs) leads to altered genomic associations with this nucleoporin.

Capturing nucleoporin-chromatin associations

NUP93 is the most abundant nucleoporin and is essential for NPC assembly and function [11]. It is a very stable and centrally located component of the NPC, giving confidence that any interactions between it and chromatin do indeed occur at the nuclear periphery rather than being due to free nucleoplasmic protein [12]. The Silver lab had previously shown by ChIP that various components of the yeast NPC, including Nic96, the yeast homolog of NUP93, could associate with specific regions of the yeast genome [13]. They have now extended this approach to regions of three different human chromosomes that interact with NUP93 in HeLa cells [10]. After cell fixation with dimethyl adipimidate and paraformaldehyde, lysis in detergent and then sonication, DNA sequences associating with NUP93 in the soluble extract were captured by ChIP, amplified, and hybridized to a tiling microarray covering chromosomes 5, 7 and 16. A total of 207 associated regions, significantly enriched in the ChIP material relative to control (input) chromatin, were defined. For some selected loci, their disposition at, or adjacent to, the nuclear periphery was confirmed by fluorescence *in situ* hybridization (FISH).

The chromosomal distribution of the NUP93-associated regions was not random. Such sites were enriched in the G-bands and depleted from the more gene-rich R-bands, consistent with the known polar organization of chromosomes in the nucleus, with G-bands tending to be concentrated at the periphery [14]. By comparison with published genome-wide distributions of histone methylation states in CD4 T cells [15], NUP93-associated regions were those typically enriched for histone H3 methylation marks that correlate with inactive chromatin (trimethylation on H3 lysine 9 (H3K9me3), lysine 27 (H3K27me3), or lysine 79 (H3K79me3)), and depleted in signatures of active genes and RNA polymerase II.

Chromatin at the nuclear periphery is generally hypoacetylated, but this can be altered by the inhibition of HDACs

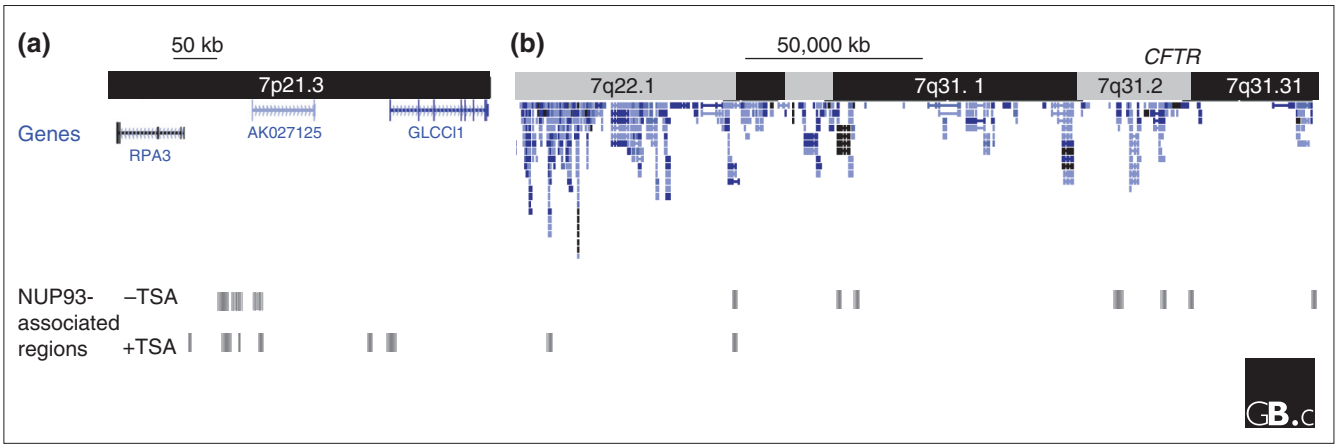

Figure 1
Effects of TSA on genomic regions associated with NUP93 in human cells. **(a)** Short-range changes in NUP93-associated regions that result from treatment with TSA (- and + TSA) are shown for a region of chromosome 7p (7p21.3). The associated regions are indicated as grey blocks in the lower part of the figure. The locations of the three genes present in 7p21.3 are indicated. Map data are from the March 2006 assembly (hg18) of the human genome. NUP93-associated sites, corresponding to the 5' and upstream regions of the gene *AK027125* in untreated controls, are broadened in TSA-treated cells to include the 5' ends of the flanking genes *RPA3* (whose expression is unchanged by TSA), and *GLCCI1* (whose expression is upregulated by TSA). **(b)** Long-range changes in NUP93-associated regions that result from treatment with TSA are shown for a region of chromosome 7q. Grey and black regions on the chromosome indicate cytogenetic bands (G-band, black; R-band, grey). The blue and black bands under the chromosome indicate the positions of genes, as indicated to the left of the figure. Map data are from the March 2006 assembly (hg18) of the human genome. Adapted from [10].

with trichostatin A (TSA) [16,17]. This treatment is sufficient to upregulate genes whose expression has been suppressed as a consequence of tethering at the nuclear periphery [7]. After treating HeLa cells for 12 hours with TSA, Brown *et al.* [10] found that NUP93 associations captured on the three analyzed chromosomes were substantially altered, with fewer (170) associated regions, indicating a dynamic shift in NPC-chromatin interactions in the presence of raised levels of histone acetylation.

Indeed, compared with the untreated cells, Brown *et al.* [10] saw an overall shift in the NUP93-associated sequences towards transcriptional start sites and regions of the genome thought to be marked with histone modifications typical of transcriptionally active regions (such as H3K4 methylation) and enriched in RNA polymerase II [15]. However, they made no analysis of chromatin structure in the TSA-treated HeLa cells, and it will be interesting to see how the histone methylation marks are altered when chromatin is hyperacetylated with TSA under the experimental conditions used in this study.

In some cases, changes in NUP93 association appear to be quite local. In one characterized example from chromosome 7, NUP93 association, previously thought to be in a purely intergenic region but actually including the 5' end of a recently annotated transcription unit (Figure 1a), then extended out to the transcription start sites of two additional flanking genes in TSA-treated cells. Expression of one of these flanking genes was upregulated in the TSA-treated cells, but the expression of the other was unaffected.

More generally, the changes in NUP93 association caused by TSA treatment seem to be long range, as the overall chromosomal distribution of associated regions shifted away from G-bands and toward R-bands (Figure 1b). This scale of genome reorganization should be detectable at the cytological level, and indeed, relocalization of loci either towards or away from the nuclear periphery was detected by FISH in TSA-treated cells. One of the loci whose association with NUP93 was diminished by TSA treatment was the cystic fibrosis transmembrane conductance regulator (*CFTR*) locus at 7q31.2 (Figure 1b). This is consistent with a previous study in which *CFTR* was observed to move away from the nuclear periphery upon TSA treatment [18]. Such large-scale spatial reorganization of the sequences at the nuclear periphery is also consistent with the changes observed in sequences interacting with the nuclear lamina in TSA-treated *Drosophila* cells [5].

Linking histone acetylation to nuclear reorganization

What might be the mechanisms by which TSA alters the association between chromatin and the nuclear periphery? Chromatin decondensation, and hence enhanced chromatin flexibility and mobility, as a consequence of the hyperacetylation induced by TSA, might account for the localized changes in NUP93-chromatin associations. However, given the constraints on chromatin motion in human cells [19], it seems unlikely that this could account for the altered long-range landscape of NUP93-associated regions after TSA treatment. Instead, it is more likely that components of the nuclear periphery have an affinity for chromatin marked

with specific histone modifications, histone variants, or other chromatin-associated proteins, and that TSA, by hyperacetylating chromatin and other proteins, modifies these interactions. In that case, the TSA-induced changes in NPC-chromatin association might well require passage through mitosis, as do other documented cases of large-scale nuclear reorganization with respect to the nuclear periphery [6,19,20].

As well as altering chromatin structure and organization, TSA also induces the nuclear redistribution of nuclear transport proteins and the histone acetyltransferase CBP, which can interact with NUP93 [21], and it is unclear what role these proteins might have in the altered NUP93-genome associations reported in response to TSA.

In budding yeast, Nic96 (NUP93) association is found with highly transcribed genes that contain the binding sites for a particular transcriptional regulator [13] and there are several examples in this organism that link the activation of gene expression with nuclear pore association [8,9]. In the current study in human cells [10] there was no statistically evident overlap between NUP93 association and genes that are either activated or repressed in response to TSA treatment.

This first report of specific genome interactions captured by ChIP with a mammalian NPC component should stimulate further investigations of the complex landscape at the nuclear periphery and the role this might play in regulating genome organization and function. Given that the diameter of the central channel through the nuclear pore is approximately the same as that of the 30-nm chromatin fiber, it seems unlikely that substantial amounts of chromatin find their way into the center of the nuclear pore. Rather, given the extent of cross-linking used before ChIP, it is likely that the associations captured by anti-NUP93 ChIP are indirect. Hence, it will be important to define exactly which components of the NPC provide the chromatin-binding domains. One way to investigate this, independently of ChIP, is the complementary approach of expressing NPC components fused to the DAM methyltransferase (DAM-ID), which has been used to identify lamin-interacting sites in *Drosophila* [5].

It will also be of interest to investigate how nuclear pore-genome associations are altered after treatment with TSA for brief periods of time (less than 4 hours) that are sufficient to induce bulk histone hyperacetylation and to relieve the transcriptional suppression of genes tethered at the nuclear membrane [7] but not long enough to allow cells to pass through mitosis or to induce large-scale relocalization of inactive chromatin away from the nuclear periphery [22]. It will also be important to establish, by specific knockdown of protein expression by short interfering RNAs, for example, whether inhibition of any particular HDAC is critical for the altered genomic association with the nuclear pores.

Finally, it remains to be resolved whether nuclear pore associations with chromatin do have a direct role in inducing gene expression in mammalian cells. The answer is likely to be as complex as the question of whether association with other components of the nuclear periphery can silence gene expression [6,7], and indeed, given the complexities of mammalian genome organization and developmentally or physiologically induced changes in gene expression, we should not expect simple black and white answers.

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